IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Zairen Sun

Serial No. : 10/067,482 Examiner: Christopher H. Yaen

Filed : February 7, 2002 Group Art Unit: 1642

Title : HUMAN DEHYDROGENASE GENE AND POLYPEPTIDE

Declaration Under 37 C.F.R. §1.132

1. I, Zairen Sun, Ph.D., am an inventor of the subject matter described and claimed in the above-identified U.S. Patent Application which is assigned to OriGene Technologies. I am also Director of Research and Development for OriGene Technologies, Inc.

2. The following experiments were performed by me, or under my supervision.

3. The results reported below were obtained using the procedures described in the Specification, e.g., on Page 2, line 28-Page 3, line 27. Briefly, Matrigel™ plug implants comprising FGF-1 were implanted subcutaneously into a host mouse. The initial bolus of FGF attracts endothelial cells into the implant, but does not result in new blood vessel formation. After about 10-15 days, the implant was re-infused with FGF-1. The FGF-1 stimulates the endothelial cells already present in the implant, initiating the process of angiogenesis. Tissue samples, removed at different intervals, were analyzed to determine their gene expression patterns.

4. Samples of the Matrigel™ plug were harvested immediately prior to the re-injection with FGF-1, and then 1 ("1h"), 8 ("8h"), and 24 ("24h") hours later. The sample removed just prior to the FGF-1 re-infusion was used to establish the basal levels of gene expression ("0 hrs") before initiation of angiogenesis.

5. Polyadenylated mRNA was isolated from each of the four samples, and used as a template for first-strand cDNA synthesis. The resulting cDNA samples were normalized using beta-actin as a standard. For the normalization procedure, PCR was performed on aliquots of the first-strand cDNA using beta-actin specific primers. The PCR products were visualized on an ethidium bromide stained agarose gel to estimate the quantity of beta-actin cDNA present in each sample. Based on these estimates, each sample was diluted with buffer until each contained the same quantity of beta-actin cDNA per unit volume.

6. To detect gene expression, PCR was carried out on aliquots of the normalized tissue samples using oligonucleotide primers specific for ANH401, ANH0058, ANH0141, and beta-actin. The reaction products were loaded on to an agarose (e.g., 1.5-2%) gel and separated electrophoretically.

The primers had the following sequences:

ANH401:	AN401F:	GGAAGATGCTGTAAGAAAACCGGT
	AN401R:	CTTGCAGTAGCTTTGTTAATTGCAC
ANH0058:	AN58F:	CAACCTTGATCCCAGCAACGTGGA
	AN58R:	GCCGTGAGCTCCAGTCCTTCCTCA
ANH0141:	AN141F:	CTGGAGTTGAACCAGGTGAAAT
	AN141R:	CAGCTTCTTCTTGGTGTTGATG
beta-actin:	MbactinF:	TGGTGGGAATGGGTCAGA
	MbactinR:	AGGGAGGAAGAGGATGCG

7. The attached figures show representative expression patterns (ethidium bromide stained agarose gels) for several differentially regulated genes identified during angiogenesis using the materials obtained according to the procedures described in Paragraph Nos. 3-5. The lane at the far left of the panel contains molecular weight standards.

8. As shown in the attachments, each of the genes has a characteristic expression pattern. ANH0058 is expressed at substantially the same levels throughout the angiogenic

process, including just prior to blood vessel formation when only endothelial cells are present. ANH0141 shows a more complex pattern, being detected before angiogenesis and again at 8 hrs, but is substantially absent at the 1 hr and 24 hr time points. ANH401 – described in the present application – is highly expressed during angiogenesis. It is expressed at very low levels immediately prior to the onset of angiogenesis, and then increases dramatically at one-hour and continues to be expressed throughout. This expression pattern makes it useful as a marker for the onset of angiogenesis, as well as for the presence or absence of blood vessels in a tumor biopsy sample.

9. I declare further that all statements made in this Declaration are of my own knowledge and are true and that all statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Date Zairen Sun